

## TITLE OF THE INVENTION

ALTERATIONS IN THE LONG QT SYNDROME GENES *KVLQT1* AND *SCN5A* AND METHODS FOR DETECTING SAME

## 5 CROSS REFERENCE TO RELATED APPLICATIONS

The present invention is related to provisional application Serial No. 60/190,057 filed 17 March 2000, and is also related to provisional application Serial No. 60/147,488 filed 9 August 1999, both of which are incorporated herein by reference.

10 This application was made with Government support from NHLBI under Grant Nos. RO1-HL46401, RO1-HL33843, RO1-HL51618, P50-HL52338 and MO1-RR000064. The federal government may have certain rights in this invention.

## BACKGROUND OF THE INVENTION

15 Long QT Syndrome (LQTS) is a cardiovascular disorder characterized by prolongation of the QT interval on electrocardiogram and presence of syncope, seizures and sudden death, usually in young, otherwise healthy individuals (Jervell and Lange-Nielsen, 1957; Romano et al., 1963; Ward, 1964). The clinical features of LQTS result from episodic ventricular tachyarrhythmias, such as *torsade de pointes* and ventricular fibrillation (Schwartz et al., 1975; 20 Moss et al., 1991). Two inherited forms of LQTS exist. The more common form, Romano-Ward syndrome (RW), is not associated with other phenotypic abnormalities and is inherited as an autosomal dominant trait with variable penetrance (Roman et al., 1963; Ward, 1964). Jervell and Lange-Nielsen syndrome (JLN) is characterized by the presence of deafness, a phenotypic abnormality inherited as an autosomal recessive trait (Jervell and Lange-Nielsen, 1957). LQTS 25 can also be acquired, usually as a result of pharmacologic therapy.

In previous studies, we mapped LQTS loci to chromosomes 11p15.5 (*LQT1*) (Keating et al., 1991), 7 q35-36 (*LQT2*) (Jiang et al., 1994) and *LQT3* to 3p21-24 (Jiang et al., 1994). A fourth locus (*LQT4*) was mapped to 4q25-27 (Schott et al., 1995). Five genes have been implicated in Romano-Ward syndrome, the autosomal dominant form of LQTS. These genes are 30 *KVLQT1* (*LQT1*) (Wang Q. et al., 1996a), *HERG* (*LQT2*) (Curran et al., 1995), *SCN5A* (*LQT3*) (Wang et al., 1995a), and two genes located at 21q22 - *KCNE1* (*LQT5*) (Splawski et al., 1997a) and *KCNE2* (*LQT6*) (Abbott et al., 1999). Mutations in *KVLQT1* and *KCNE1* also cause the

Jervell and Lange-Nielsen syndrome, a form of LQTS associated with deafness, a phenotypic abnormality inherited in an autosomal recessive fashion.

*KVLQT1*, *HERG*, *KCNE1* and *KCNE2* encode potassium channel subunits. Four *KVLQT1*  $\alpha$ -subunits assemble with minK ( $\beta$ -subunits encoded by *KCNE1*, stoichiometry is unknown) to form  $I_{Ks}$  channels underlying the slowly activating delayed rectifier potassium current in the heart (Sanguinetti et al., 1996a; Barhanin et al., 1996). Four *HERG*  $\alpha$ -subunits assemble with MiRP1 (encoded by *KCNE2*, stoichiometry unknown) to form  $I_{Kr}$  channels, which underlie the rapidly activating, delayed rectifier potassium current (Abbott et al., 1999). Mutant subunits lead to reduction of  $I_{Ks}$  or  $I_{Kr}$  by a loss-of-function mechanism, often with a dominant-negative effect (Chouabe et al., 1997; Shalaby et al., 1997; Wollnik et al., 1997; Sanguinetti et al., 1996b). *SCN5A* encodes the cardiac sodium channel that is responsible for  $I_{Na}$ , the sodium current in the heart (Gellens et al., 1992). LQTS-associated mutations in *SCN5A* cause a gain-of-function (Bennett et al., 1995; Dumaine et al., 1996). In the heart, reduced  $I_{Ks}$  or  $I_{Kr}$  or increased  $I_{Na}$  leads to prolongation of the cardiac action potential, lengthening of the QT interval and increased risk of arrhythmia. *KVLQT1* and *KCNE1* are also expressed in the inner ear (Neyroud et al., 1997; Vetter et al., 1996). Others and we demonstrated that complete loss of  $I_{Ks}$  causes the severe cardiac phenotype and deafness in JLN (Neyroud et al., 1997; Splawski et al., 1997b; Tyson et al., 1997; Schulze-Bahr et al., 1997).

Presymptomatic diagnosis of LQTS is currently based on prolongation of the QT interval on electrocardiogram. Genetic studies, however, have shown that diagnosis based solely on electrocardiogram is neither sensitive nor specific (Vincent et al., 1992; Priori et al., 1999). Genetic screening using mutational analysis can improve presymptomatic diagnosis. However, a comprehensive study identifying and cataloging all LQTS-associated mutations in all five genes has not been achieved. To determine the relative frequency of mutations in each gene, facilitate presymptomatic diagnosis and enable genotype-phenotype studies, we screened a pool of 262 unrelated individuals with LQTS for mutations in the five defined genes. The results of these studies are presented in the Examples below.

The present invention relates to alterations in the *KVLQT1*, *HERG*, *SCN5A*, *KCNE1* and *KCNE2* genes and methods for detecting such alterations.

The publications and other materials used herein to illuminate the background of the invention or provide additional details respecting the practice, are incorporated by reference, and for convenience are respectively grouped in the appended List of References.

The present invention is directed to alterations in genes and gene products associated with long QT syndrome and to a process for the diagnosis and prevention of LQTS. LQTS is diagnosed in accordance with the present invention by analyzing the DNA sequence of the *KVLQT1*, *HERG*, *SCN5A*, *KCNE1* or *KCNE2* gene of an individual to be tested and comparing the respective DNA sequence to the known DNA sequence of the normal gene. Alternatively, these genes of an individual to be tested can be screened for mutations which cause LQTS. Prediction of LQTS will enable practitioners to prevent this disorder using existing medical therapy.

#### SUMMARY OF THE INVENTION

The present invention relates to alterations in the *KVLQT1*, *HERG*, *SCN5A*, *KCNE1* and *KCNE2* genes and methods for detecting such alterations. The alterations in the *KVLQT1*, *HERG*, *SCN5A*, *KCNE1* and *KCNE2* genes include mutations and polymorphisms. Included among the mutations are frameshift, nonsense, splice, regulatory and missense mutations. Any method which is capable of detecting the alterations described herein can be used. Such methods include, but are not limited to, DNA sequencing, allele-specific probing, mismatch detection, single stranded conformation polymorphism detection and allele-specific PCR amplification.

#### BRIEF DESCRIPTION OF THE FIGURES

Figure 1 is a schematic representation of the predicted topology of *KVLQT1* and the locations of LQTS-associated mutations. *KVLQT1* consists of six putative transmembrane segments (S1 to S6) and a pore (Pore) region. Each circle represents an amino acid. The approximate location of LQTS-associated mutations identified in our laboratory are shown with filled circles.

Figure 2 is a schematic representation of *HERG* mutations. *HERG* consists of six putative transmembrane segments (S1 to S6) and a pore (Pore) region. Location of LQTS-associated mutations are shown with filled circles.

Figure 3 is a schematic representation of SCN5A and locations of LQTS-associated mutations. SCN5A consists of four domain (DI to DIV), each of which has six putative transmembrane segments (S1 to S6) and a pore (Pore) region. Location of LQTS-associated mutations identified in our laboratory are shown with filled circles.

Figure 4 is a schematic representation of minK and locations of LQT-associated mutations. MinK consists of one putative transmembrane domain (S1). The approximate location of LQTS-associated mutations identified in our laboratory are shown with filled circles.

Figure 5 is a schematic representation of the predicted topology of MiRP1 and locations of arrhythmia-associated mutations. MiRP1 consists of one putative transmembrane domain (S1). The approximate location of arrhythmia-associated mutations identified in our laboratory are shown with filled circles.

#### DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to alterations in the *KVLQT1*, *HERG*, *SCN5A*, *KCNE1* and *KCNE2* genes and methods for detecting such alterations. The alterations in the *KVLQT1*, *HERG*, *SCN5A*, *KCNE1* and *KCNE2* genes include mutations and polymorphisms. Included among the mutations are frameshift, nonsense, splice, regulatory and missense mutations. Any method which is capable of detecting the mutations and polymorphisms described herein can be used. Such methods include, but are not limited to, DNA sequencing, allele-specific probing, mismatch detection, single stranded conformation polymorphism detection and allele-specific PCR amplification.

*KVLQT1*, *HERG*, *SCN5A*, *KCNE1* and *KCNE2* mutations cause increased risk for LQTS. Many different mutations occur in *KVLQT1*, *HERG*, *SCN5A*, *KCNE1* and *KCNE2*. In order to detect the presence of alterations in the *KVLQT1*, *HERG*, *SCN5A*, *KCNE1* and *KCNE2* genes, a biological sample such as blood is prepared and analyzed for the presence or absence of a given alteration of *KVLQT1*, *HERG*, *SCN5A*, *KCNE1* or *KCNE2*. In order to detect the increased risk for LQTS or for the lack of such increased risk, a biological sample is prepared and analyzed for the presence or absence of a mutant allele of *KVLQT1*, *HERG*, *SCN5A*, *KCNE1* or *KCNE2*. Results of these tests and interpretive information are returned to the health care provider for communication to the tested individual. Such diagnoses may be performed by diagnostic

laboratories or, alternatively, diagnostic kits are manufactured and sold to health care providers or to private individuals for self-diagnosis.

The presence of hereditary LQTS may be ascertained by testing any tissue of a human for mutations of the *KVLQT1*, *HERG*, *SCN5A*, *KCNE1* or *KCNE2* gene. For example, a person who has inherited a germline *HERG* mutation would be prone to develop LQTS. This can be determined by testing DNA from any tissue of the person's body. Most simply, blood can be drawn and DNA extracted from the cells of the blood. In addition, prenatal diagnosis can be accomplished by testing fetal cells, placental cells or amniotic cells for mutations of the *KVLQT1*, *HERG*, *SCN5A*, *KCNE1* or *KCNE2* gene. Alteration of a wild-type *KVLQT1*, *HERG*, *SCN5A*, *KCNE1* or *KCNE2* allele, whether, for example, by point mutation or deletion, can be detected by any of the means discussed herein.

There are several methods that can be used to detect DNA sequence variation. Direct DNA sequencing, either manual sequencing or automated fluorescent sequencing can detect sequence variation. Another approach is the single-stranded conformation polymorphism assay (SSCP) (Orita et al., 1989). This method does not detect all sequence changes, especially if the DNA fragment size is greater than 200 bp, but can be optimized to detect most DNA sequence variation. The reduced detection sensitivity is a disadvantage, but the increased throughput possible with SSCP makes it an attractive, viable alternative to direct sequencing for mutation detection on a research basis. The fragments which have shifted mobility on SSCP gels are then sequenced to determine the exact nature of the DNA sequence variation. Other approaches based on the detection of mismatches between the two complementary DNA strands include clamped denaturing gel electrophoresis (CDGE) (Sheffield et al., 1991), heteroduplex analysis (HA) (White et al., 1992) and chemical mismatch cleavage (CMC) (Grompe et al., 1989). None of the methods described above will detect large deletions, duplications or insertions, nor will they detect a regulatory mutation which affects transcription or translation of the protein. Other methods which might detect these classes of mutations such as a protein truncation assay or the asymmetric assay, detect only specific types of mutations and would not detect missense mutations. A review of currently available methods of detecting DNA sequence variation can be found in a recent review by Grompe (1993). Once a mutation is known, an allele specific detection approach such as allele specific oligonucleotide (ASO) hybridization can be utilized to rapidly screen large numbers of other samples for that same mutation. Such a technique can

utilize probes which are labeled with gold nanoparticles to yield a visual color result (Elghanian et al., 1997).

A rapid preliminary analysis to detect polymorphisms in DNA sequences can be performed by looking at a series of Southern blots of DNA cut with one or more restriction enzymes, preferably with a large number of restriction enzymes. Each blot contains a series of normal individuals and a series of LQTS cases. Southern blots displaying hybridizing fragments (differing in length from control DNA when probed with sequences near or including the *HERG* locus) indicate a possible mutation. If restriction enzymes which produce very large restriction fragments are used, then pulsed field gel electrophoresis (PFGE) is employed.

Detection of point mutations may be accomplished by molecular cloning of the *KVLQT1*, *HERG*, *SCN5A*, *KCNE1* or *KCNE2* alleles and sequencing the alleles using techniques well known in the art. Also, the gene or portions of the gene may be amplified, e.g., by PCR or other amplification technique, and the amplified gene or amplified portions of the gene may be sequenced.

There are six well known methods for a more complete, yet still indirect, test for confirming the presence of a susceptibility allele: 1) single stranded conformation analysis (SSCP) (Orita et al., 1989); 2) denaturing gradient gel electrophoresis (DGGE) (Wartell et al., 1990; Sheffield et al., 1989); 3) RNase protection assays (Finkelstein et al., 1990; Kinszler et al., 1991); 4) allele-specific oligonucleotides (ASOs) (Conner et al., 1983); 5) the use of proteins which recognize nucleotide mismatches, such as the *E. coli* mutS protein (Modrich, 1991); and 6) allele-specific PCR (Ruano and Kidd, 1989). For allele-specific PCR, primers are used which hybridize at their 3' ends to a particular *KVLQT1*, *HERG*, *SCN5A*, *KCNE1* or *KCNE2* mutation. If the particular mutation is not present, an amplification product is not observed. Amplification Refractory Mutation System (ARMS) can also be used, as disclosed in European Patent Application Publication No. 0332435 and in Newton et al., 1989. Insertions and deletions of genes can also be detected by cloning, sequencing and amplification. In addition, restriction fragment length polymorphism (RFLP) probes for the gene or surrounding marker genes can be used to score alteration of an allele or an insertion in a polymorphic fragment. Such a method is particularly useful for screening relatives of an affected individual for the presence of the mutation found in that individual. Other techniques for detecting insertions and deletions as known in the art can be used.

In the first three methods (SSCP, DGGE and RNase protection assay), a new electrophoretic band appears. SSCP detects a band which migrates differentially because the sequence change causes a difference in single-strand, intramolecular base pairing. RNase protection involves cleavage of the mutant polynucleotide into two or more smaller fragments. DGGE detects differences in migration rates of mutant sequences compared to wild-type sequences, using a denaturing gradient gel. In an allele-specific oligonucleotide assay, an oligonucleotide is designed which detects a specific sequence, and the assay is performed by detecting the presence or absence of a hybridization signal. In the mutS assay, the protein binds only to sequences that contain a nucleotide mismatch in a heteroduplex between mutant and wild-type sequences.

Mismatches, according to the present invention, are hybridized nucleic acid duplexes in which the two strands are not 100% complementary. Lack of total homology may be due to deletions, insertions, inversions or substitutions. Mismatch detection can be used to detect point mutations in the gene or in its mRNA product. While these techniques are less sensitive than sequencing, they are simpler to perform on a large number of samples. An example of a mismatch cleavage technique is the RNase protection method. In the practice of the present invention, the method involves the use of a labeled riboprobe which is complementary to the human wild-type *KVLQT1*, *HERG*, *SCN5A*, *KCNE1* or *KCNE2* gene coding sequence. The riboprobe and either mRNA or DNA isolated from the person are annealed (hybridized) together and subsequently digested with the enzyme RNase A which is able to detect some mismatches in a duplex RNA structure. If a mismatch is detected by RNase A, it cleaves at the site of the mismatch. Thus, when the annealed RNA preparation is separated on an electrophoretic gel matrix, if a mismatch has been detected and cleaved by RNase A, an RNA product will be seen which is smaller than the full length duplex RNA for the riboprobe and the mRNA or DNA. The riboprobe need not be the full length of the mRNA or gene but can be a segment of either. If the riboprobe comprises only a segment of the mRNA or gene, it will be desirable to use a number of these probes to screen the whole mRNA sequence for mismatches.

In similar fashion, DNA probes can be used to detect mismatches, through enzymatic or chemical cleavage. See, e.g., Cotton et al., 1988; Shenk et al., 1975; Novack et al., 1986. Alternatively, mismatches can be detected by shifts in the electrophoretic mobility of mismatched duplexes relative to matched duplexes. See, e.g., Cariello, 1988. With either riboprobes or DNA

probes, the cellular mRNA or DNA which might contain a mutation can be amplified using PCR (see below) before hybridization. Changes in DNA of the *KVLQT1*, *HERG*, *SCN5A*, *KCNE1* or *KCNE2* gene can also be detected using Southern hybridization, especially if the changes are gross rearrangements, such as deletions and insertions.

5 DNA sequences of the *KVLQT1*, *HERG*, *SCN5A*, *KCNE1* or *KCNE2* gene which have been amplified by use of PCR may also be screened using allele-specific probes. These probes are nucleic acid oligomers, each of which contains a region of the gene sequence harboring a known mutation. For example, one oligomer may be about 30 nucleotides in length, corresponding to a portion of the gene sequence. By use of a battery of such allele-specific  
10 probes, PCR amplification products can be screened to identify the presence of a previously identified mutation in the gene. Hybridization of allele-specific probes with amplified *KVLQT1*, *HERG*, *SCN5A*, *KCNE1* or *KCNE2* sequences can be performed, for example, on a nylon filter. Hybridization to a particular probe under high stringency hybridization conditions indicates the presence of the same mutation in the tissue as in the allele-specific probe.

15 The newly developed technique of nucleic acid analysis via microchip technology is also applicable to the present invention. In this technique, literally thousands of distinct oligonucleotide probes are built up in an array on a silicon chip. Nucleic acid to be analyzed is fluorescently labeled and hybridized to the probes on the chip. It is also possible to study nucleic acid-protein interactions using these nucleic acid microchips. Using this technique one can  
20 determine the presence of mutations or even sequence the nucleic acid being analyzed or one can measure expression levels of a gene of interest. The method is one of parallel processing of many, even thousands, of probes at once and can tremendously increase the rate of analysis. Several papers have been published which use this technique. Some of these are Hacia et al., 1996; Shoemaker et al., 1996; Chee et al., 1996; Lockhart et al., 1996; DeRisi et al., 1996;  
25 Lipshutz et al., 1995. This method has already been used to screen people for mutations in the breast cancer gene *BRCA1* (Hacia et al., 1996). This new technology has been reviewed in a news article in Chemical and Engineering News (Borman, 1996) and been the subject of an editorial (Editorial, Nature Genetics, 1996). Also see Fodor (1997).

30 The most definitive test for mutations in a candidate locus is to directly compare genomic *KVLQT1*, *HERG*, *SCN5A*, *KCNE1* or *KCNE2* sequences from patients with those from a control



population. Alternatively, one could sequence messenger RNA after amplification, e.g., by PCR, thereby eliminating the necessity of determining the exon structure of the candidate gene.

Mutations from patients falling outside the coding region of *KVLQT1*, *HERG*, *SCN5A*, *KCNE1* or *KCNE2* can be detected by examining the non-coding regions, such as introns and regulatory sequences near or within the genes. An early indication that mutations in noncoding regions are important may come from Northern blot experiments that reveal messenger RNA molecules of abnormal size or abundance in patients as compared to control individuals.

Alteration of *KVLQT1*, *HERG*, *SCN5A*, *KCNE1* or *KCNE2* mRNA expression can be detected by any techniques known in the art. These include Northern blot analysis, PCR amplification and RNase protection. Diminished mRNA expression indicates an alteration of the wild-type gene. Alteration of wild-type genes can also be detected by screening for alteration of wild-type *KVLQT1*, *HERG*, *SCN5A*, *KCNE1* or *KCNE2* protein. For example, monoclonal antibodies immunoreactive with *HERG* can be used to screen a tissue. Lack of cognate antigen would indicate a mutation. Antibodies specific for products of mutant alleles could also be used to detect mutant gene product. Such immunological assays can be done in any convenient formats known in the art. These include Western blots, immunohistochemical assays and ELISA assays. Any means for detecting an altered *KVLQT1*, *HERG*, *SCN5A*, *KCNE1* or *KCNE2* protein can be used to detect alteration of wild-type *KVLQT1*, *HERG*, *SCN5A*, *KCNE1* or *KCNE2* genes. Functional assays, such as protein binding determinations, can be used. In addition, assays can be used which detect *KVLQT1*, *HERG*, *SCN5A*, *KCNE1* or *KCNE2* biochemical function. Finding a mutant *KVLQT1*, *HERG*, *SCN5A*, *KCNE1* or *KCNE2* gene product indicates alteration of a wild-type *KVLQT1*, *HERG*, *SCN5A*, *KCNE1* or *KCNE2* gene.

Mutant *KVLQT1*, *HERG*, *SCN5A*, *KCNE1* or *KCNE2* genes or gene products can also be detected in other human body samples, such as serum, stool, urine and sputum. The same techniques discussed above for detection of mutant genes or gene products in tissues can be applied to other body samples. By screening such body samples, a simple early diagnosis can be achieved for hereditary LQTS.

Initially, the screening method involves amplification of the relevant *KVLQT1*, *HERG*, *SCN5A*, *KCNE1* or *KCNE2* sequence. In another preferred embodiment of the invention, the screening method involves a non-PCR based strategy. Such screening methods include two-step label amplification methodologies that are well known in the art. Both PCR and non-PCR based

screening strategies can detect target sequences with a high level of sensitivity. Further details of these methods are briefly presented below and further descriptions can be found in PCT published application WO 96/05306, incorporated herein by reference.

The most popular method used today is target amplification. Here, the target nucleic acid sequence is amplified with polymerases. One particularly preferred method using polymerase-driven amplification is the polymerase chain reaction (PCR). The polymerase chain reaction and other polymerase-driven amplification assays can achieve over a million-fold increase in copy number through the use of polymerase-driven amplification cycles. Once amplified, the resulting nucleic acid can be sequenced or used as a substrate for DNA probes.

When the probes are used to detect the presence of the target sequences, the biological sample to be analyzed, such as blood or serum, may be treated, if desired, to extract the nucleic acids. The sample nucleic acid may be prepared in various ways to facilitate detection of the target sequence; e.g. denaturation, restriction digestion, electrophoresis or dot blotting. The targeted region of the analyte nucleic acid usually must be at least partially single-stranded to form hybrids with the targeting sequence of the probe. If the sequence is naturally single-stranded, denaturation will not be required. However, if the sequence is double-stranded, the sequence will probably need to be denatured. Denaturation can be carried out by various techniques known in the art.

Analyte nucleic acid and probe are incubated under conditions which promote stable hybrid formation of the target sequence in the probe with the putative targeted sequence in the analyte. The region of the probes which is used to bind to the analyte can be made completely complementary to the targeted region of the genes. Therefore, high stringency conditions are desirable in order to prevent false positives. However, conditions of high stringency are used only if the probes are complementary to regions of the chromosome which are unique in the genome. The stringency of hybridization is determined by a number of factors during hybridization and during the washing procedure, including temperature, ionic strength, base composition, probe length, and concentration of formamide. Under certain circumstances, the formation of higher order hybrids, such as triplexes, quadruplexes, etc., may be desired to provide the means of detecting target sequences.

Detection, if any, of the resulting hybrid is usually accomplished by the use of labeled probes. Alternatively, the probe may be unlabeled, but may be detectable by specific binding

with a ligand which is labeled, either directly or indirectly. Suitable labels, and methods for labeling probes and ligands are known in the art, and include, for example, radioactive labels which may be incorporated by known methods (e.g., nick translation, random priming or kinasing), biotin, fluorescent groups, chemiluminescent groups (e.g., dioxetanes, particularly triggered dioxetanes), enzymes, antibodies and the like. Variations of this basic scheme known in the art, and include those variations that facilitate separation of the hybrids to be detected from extraneous materials and/or that amplify the signal from the labeled moiety. A number of these variations are well known.

As noted above, non-PCR based screening assays are also contemplated in this invention.

This procedure hybridizes a nucleic acid probe (or an analog such as a methyl phosphonate backbone replacing the normal phosphodiester), to the low level DNA target. This probe may have an enzyme covalently linked to the probe, such that the covalent linkage does not interfere with the specificity of the hybridization. This enzyme-probe-conjugate-target nucleic acid complex can then be isolated away from the free probe enzyme conjugate and a substrate is added for enzyme detection. Enzymatic activity is observed as a change in color development or luminescent output resulting in a  $10^3$ - $10^6$  increase in sensitivity. For example, the preparation of oligodeoxynucleotide-alkaline phosphatase conjugates and their use as hybridization probes are well known.

Two-step label amplification methodologies are known in the art. These assays work on the principle that a small ligand (such as digoxigenin, biotin, or the like) is attached to a nucleic acid probe capable of specifically binding the target gene. Allele specific probes are also contemplated within the scope of this example.

In one example, the small ligand attached to the nucleic acid probe is specifically recognized by an antibody-enzyme conjugate. In one embodiment of this example, digoxigenin is attached to the nucleic acid probe. Hybridization is detected by an antibody-alkaline phosphatase conjugate which turns over a chemiluminescent substrate. In a second example, the small ligand is recognized by a second ligand-enzyme conjugate that is capable of specifically complexing to the first ligand. A well known embodiment of this example is the biotin-avidin type of interactions. Methods for labeling nucleic acid probes and their use in biotin-avidin based assays are well known.

It is also contemplated within the scope of this invention that the nucleic acid probe assays of this invention will employ a cocktail of nucleic acid probes capable of detecting the gene or genes. Thus, in one example to detect the presence of *KVLQT1* in a cell sample, more than one probe complementary to *KVLQT1* is employed and in particular the number of different probes is alternatively 2, 3, or 4 different nucleic acid probe sequences. In another example, to detect the presence of mutations in the *KVLQT1* gene sequence in a patient, more than one probe complementary to *KVLQT1* is employed where the cocktail includes probes capable of binding to the allele-specific mutations identified in populations of patients with alterations in *KVLQT1*. In this embodiment, any number of probes can be used.

Large amounts of the polynucleotides of the present invention may be produced by replication in a suitable host cell. Natural or synthetic polynucleotide fragments coding for a desired fragment will be incorporated into recombinant polynucleotide constructs, usually DNA constructs, capable of introduction into and replication in a prokaryotic or eukaryotic cell. Usually the polynucleotide constructs will be suitable for replication in a unicellular host, such as yeast or bacteria, but may also be intended for introduction to (with and without integration within the genome) cultured mammalian or plant or other eukaryotic cell lines. The purification of nucleic acids produced by the methods of the present invention are described, e.g., in Sambrook et al., 1989 or Ausubel et al., 1992.

The polynucleotides of the present invention may also be produced by chemical synthesis, e.g., by the phosphoramidite method described by Beaucage and Caruthers (1981) or the triester method according to Matteucci and Caruthers (1981) and may be performed on commercial, automated oligonucleotide synthesizers. A double-stranded fragment may be obtained from the single-stranded product of chemical synthesis either by synthesizing the complementary strand and annealing the strand together under appropriate conditions or by adding the complementary strand using DNA polymerase with an appropriate primer sequence.

Polynucleotide constructs prepared for introduction into a prokaryotic or eukaryotic host may comprise a replication system recognized by the host, including the intended polynucleotide fragment encoding the desired polypeptide, and will preferably also include transcription and translational initiation regulatory sequences operably linked to the polypeptide encoding segment. Expression vectors may include, for example, an origin of replication or autonomously replicating sequence (ARS) and expression control sequences, a promoter, an enhancer and

necessary processing information sites, such as ribosome-binding sites, RNA splice sites, polyadenylation sites, transcriptional terminator sequences, and mRNA stabilizing sequences. Such vectors may be prepared by means of standard recombinant techniques well known in the art and discussed, for example, in Sambrook et al. (1989) or Ausubel et al. (1992).

5           An appropriate promoter and other necessary vector sequences will be selected so as to be functional in the host, and may include, when appropriate, those naturally associated with the *KVLQT1* or other gene. Examples of workable combinations of cell lines and expression vectors are described in Sambrook et al. (1989) or Ausubel et al. (1992); see also, e.g., Metzger et al. (1988). Many useful vectors are known in the art and may be obtained from such vendors as  
10   Stratagene, New England Biolabs, Promega Biotech, and others. Promoters such as the trp, lac and phage promoters, tRNA promoters and glycolytic enzyme promoters may be used in prokaryotic hosts. Useful yeast promoters include promoter regions for metallothionein, 3-phosphoglycerate kinase or other glycolytic enzymes such as enolase or glyceraldehyde-3-phosphate dehydrogenase, enzymes responsible for maltose and galactose utilization, and others.  
15   Vectors and promoters suitable for use in yeast expression are further described in Hitzeman et al., EP 73,675A. Appropriate non-native mammalian promoters might include the early and late promoters from SV40 (Fiers et al., 1978) or promoters derived from murine Molony leukemia virus, mouse tumor virus, avian sarcoma viruses, adenovirus II, bovine papilloma virus or polyoma. Insect promoters may be derived from baculovirus. In addition, the construct may be  
20   joined to an amplifiable gene (e.g., DHFR) so that multiple copies of the gene may be made. For appropriate enhancer and other expression control sequences, see also Enhancers and Eukaryotic Gene Expression, Cold Spring Harbor Press, Cold Spring Harbor, New York (1983). See also, e.g., U.S. Patent Nos. 5,691,198; 5,735,500; 5,747,469 and 5,436,146.

25           While such expression vectors may replicate autonomously, they may also replicate by being inserted into the genome of the host cell, by methods well known in the art.

          Expression and cloning vectors will likely contain a selectable marker, a gene encoding a protein necessary for survival or growth of a host cell transformed with the vector. The presence of this gene ensures growth of only those host cells which express the inserts. Typical selection genes encode proteins that a) confer resistance to antibiotics or other toxic substances,  
30   e.g. ampicillin, neomycin, methotrexate, etc., b) complement auxotrophic deficiencies, or c) supply critical nutrients not available from complex media, e.g., the gene encoding D-alanine

racemase for *Bacilli*. The choice of the proper selectable marker will depend on the host cell, and appropriate markers for different hosts are well known in the art.

The vectors containing the nucleic acids of interest can be transcribed *in vitro*, and the resulting RNA introduced into the host cell by well-known methods, e.g., by injection (see, Kubo et al. (1988)), or the vectors can be introduced directly into host cells by methods well known in the art, which vary depending on the type of cellular host, including electroporation; transfection employing calcium chloride, rubidium chloride calcium phosphate, DEAE-dextran, or other substances; microprojectile bombardment; lipofection; infection (where the vector is an infectious agent, such as a retroviral genome); and other methods. See generally, Sambrook et al. (1989) and Ausubel et al. (1992). The introduction of the polynucleotides into the host cell by any method known in the art, including, *inter alia*, those described above, will be referred to herein as "transformation." The cells into which have been introduced nucleic acids described above are meant to also include the progeny of such cells.

Large quantities of the nucleic acids and polypeptides of the present invention may be prepared by expressing the *KVLQT1* nucleic acid or portions thereof in vectors or other expression vehicles in compatible prokaryotic or eukaryotic host cells. The most commonly used prokaryotic hosts are strains of *Escherichia coli*, although other prokaryotes, such as *Bacillus subtilis* or *Pseudomonas* may also be used.

Mammalian or other eukaryotic host cells, such as those of yeast, filamentous fungi, plant, insect, or amphibian or avian species, may also be useful for production of the proteins of the present invention. Propagation of mammalian cells in culture is *per se* well known. See, Jakoby and Pastan (eds.) (1979). Examples of commonly used mammalian host cell lines are VERO and HeLa cells, Chinese hamster ovary (CHO) cells, and WI38, BHK, and COS cell lines, although it will be appreciated by the skilled practitioner that other cell lines may be appropriate, e.g., to provide higher expression, desirable glycosylation patterns, or other features. An example of a commonly used insect cell line is SF9.

Clones are selected by using markers depending on the mode of the vector construction. The marker may be on the same or a different DNA molecule, preferably the same DNA molecule. In prokaryotic hosts, the transformant may be selected, e.g., by resistance to ampicillin, tetracycline or other antibiotics. Production of a particular product based on temperature sensitivity may also serve as an appropriate marker.

Prokaryotic or eukaryotic cells transformed with the polynucleotides of the present invention will be useful not only for the production of the nucleic acids and polypeptides of the present invention, but also, for example, in studying the characteristics of KVLQT1 or other polypeptides.

5       The probes and primers based on the *KVLQT1* or other gene sequences disclosed herein are used to identify homologous *KVLQT1* or other gene sequences and proteins in other species. These gene sequences and proteins are used in the diagnostic/prognostic, therapeutic and drug screening methods described herein for the species from which they have been isolated.

10       The studies described in the Examples below resulted in the determination of many novel mutations. Previous studies had defined 126 distinct disease causing mutations in the LQTS genes *KVLQT1*, *HERG*, *SCN5A*, *KCNE1* and *KCNE2* (Wang Q. et al., 1996a; Curran et al., 1995; Wang et al., 1995a; Splawski et al., 1997a; Abbott et al., 1999; Chouabe et al., 1997; Wollnik et al., 1997; Neyroud et al., 1997; Splawski et al., 1997b; Tyson et al., 1997; Schulze-Bahr et al., 1997; Priori et al., 1999; Splawski et al., 1998; Wang et al., 1995b; Russell et al., 1996; Neyroud et al., 1998; Neyroud et al., 1999; Donger et al., 1997; Tanaka et al., 1997; Jongbloed et al., 1999; Priori et al., 1998; Itoh et al., 1998a; Itoh et al., 1998b; Mohammad-Panah et al., 1999; Saarinen et al., 1998; Ackerman et al., 1998; Berthet et al., 1999; Kanters, 1998; van den Berg et al., 1997; Dausse et al., 1996; Benson et al., 1996; Akimoto et al., 1998; Satler et al., 1996; Satler et al., 1998; Makita et al., 1998; An et al., 1998; Schulze-Bahr et al., 1995; Duggal et al., 1998; Chen Q. et al., 1999; Li et al., 1998; Wei et al., 1999; Larsen et al., 1999a; Bianchi et al., 1999; Ackerman et al., 1999a; Ackerman et al., 1999b; Murray et al., 1999; Larsen et al., 1999b; Yoshida et al., 1999; Wattanasirichaigoon et al., 1999; Bezzina et al., 1999; Hoorntje et al., 1999). The sequence of each wild-type gene has been published. The *KVLQT1* can be found in Splawski et al. (1998) and the coding region of the cDNA is shown herein as SEQ ID NO:1 and the encoded KVLQT1 is shown as SEQ ID NO:2. *SCN5A* was reported by Gellens et al. (1992) and its sequence is provided by GenBank Accession No. NM\_000335. The coding sequence of *SCN5A* is shown herein as SEQ ID NO:3 and the encoded *SCN5A* is shown as SEQ ID NO:4. Most of the mutations were found in *KVLQT1* (Yoshida et al., 1999) and *HERG* (Itoh et al., 1998b), and fewer in *SCN5A* (Wang Q. et al., 1996a), *KCNE1* (Jiang et al., 1994) and *KCNE2* (Ward, 1964). These mutations were identified in regions with known intron/exon structure, primarily the transmembrane and pore domains. In this study, we screened 262 individuals with

LQTS for mutations in all known arrhythmia genes. We identified 134 mutations, 80 of which were novel. Together with 43 mutations reported in our previous studies, we have now identified 177 mutations in these 262 LQTS individuals (68%). The failure to identify mutations in 32% of the individuals may result from phenotypic errors, incomplete sensitivity of SSCP or presence of mutations in regulatory sequences. However, it is also clear that additional LQTS genes await discovery (Jiang et al., 1994; Schott et al., 1995).

Missense mutations were most common (72%), followed by frameshift mutations (10%), in-frame deletions, nonsense and splice site mutations (5-7% each). Most mutations resided in intracellular (52%) and transmembrane (30%) domains; 12% were found in pore and 6% in extracellular segments. One hundred one of the 129 distinct LQTS mutations (78%) were identified in single families or individuals. Most of the 177 mutations were found in *KVLQT1* (75 or 42%) and *HERG* (80 or 45%). These two genes accounted for 87% of the identified mutations, while mutations in *SCN5A* (14 or 8%), *KCNE1* (5 or 3%) and *KCNE2* (3 or 2%) accounted for the other 13%.

Multiple mutations were found in regions encoding S5, S5/P, P and S6 of *KVLQT1* and *HERG*. The P region of potassium channels forms the outer pore and contains the selectivity filter (Doyle et al., 1998). Transmembrane segment 6, corresponding to the inner helix of KcsA, forms the inner 2/3 of the pore. This structure is supported by the S5 transmembrane segment, corresponding to the outer helix of KcsA, and is conserved from prokaryotes to eukaryotes ((MacKinnon et al., 1998). Mutations in these regions will likely disrupt potassium transport. Many mutations were identified in the C-termini of *KVLQT1* and *HERG*. Changes in the C-terminus of *HERG* could lead to anomalies in tetramerization as it has been proposed that the C-terminus of eag, which is related to *HERG*, is involved in this process (Ludwig et al., 1994).

Multiple mutations were also identified in regions that were different for *KVLQT1* and *HERG*. In *KVLQT1*, multiple mutations were found in the sequences coding for the S2/S3 and S4/S5 linkers. Coexpression of S2/S3 mutants with wild-type *KVLQT1* in *Xenopus* oocytes led to simple loss of function or dominant-negative effect without significantly changing the biophysical properties of  $I_{Ks}$  channels (Chouabe et al., 1997; Shalaby et al., 1997; Wang et al., 1999). On the other hand, S4/S5 mutations altered the gating properties of the channels and modified *KVLQT1* interactions with minK subunits (Wang et al., 1999; Franqueza et al., 1999).



In *HERG*, more than 20 mutations were identified in the N-terminus. *HERG* channels lacking this region deactivate faster and mutations in the region had a similar effect (Chen J. et al., 1999).

Mutations in *KCNE1* and *KCNE2*, encoding minK and MiRP1, the respective  $I_{Ks}$  and  $I_{Kr}$   $\beta$ -subunits, altered the biophysical properties of the channels (Splawski et al., 1997a; Abbott et al., 1999; Sesti and Goldstein, 1998). A MiRP1 mutant, involved in clarithromycin-induced arrhythmia, increased channel blockade by the antibiotic (Abbott et al., 1999). Mutations in *SCN5A*, the sodium channel  $\alpha$ -subunit responsible for cardiac  $I_{Na}$ , destabilized the inactivation gate causing delayed channel inactivation and dispersed reopenings (Bennett et al., 1995; Dumaine et al., 1996; Wei et al., 1999; Wang DW et al., 1996). One *SCN5A* mutant affected the interactions with the sodium channel  $\beta$ -subunit (An et al., 1998).

It is interesting to note that probands with *KCNE1* and *KCNE2* mutations were older and had shorter QTc than probands with the other genotypes. The significance of these differences is unknown, however, as the number of probands with *KCNE1* and *KCNE2* genotypes was small.

This catalogue of mutations will facilitate genotype-phenotype analyses. It also has clinical implications for presymptomatic diagnosis and, in some cases, for therapy. Patients with mutations in *KVLQT1*, *HERG*, *KCNE1* and *KCNE2*, for example, may benefit from potassium therapy (Compton et al., 1996). Sodium channel blockers, on the other hand, might be helpful in patients with *SCN5A* mutations (Schwartz et al. (1995). The identification of mutations is of importance for ion channel studies as well. The expression of mutant channels in heterologous systems can reveal how structural changes influence the behavior of the channel or how mutations affect processing (Zhou et al., 1998; Furutani et al., 1999). These studies improve our understanding of channel function and provide insights into mechanisms of disease. Finally, mutation identification will contribute to the development of genetic screening for arrhythmia susceptibility.

The present invention is described by reference to the following Examples, which are offered by way of illustration and are not intended to limit the invention in any manner. Standard techniques well known in the art or the techniques specifically described in the Examples were utilized.

Example 1Ascertainment and Phenotyping

Individuals were ascertained in clinics from North America and Europe. Individuals were evaluated for LQTS based on QTc (the QT interval corrected for heart rate) and for the presence of symptoms. In this study, we focused on the probands. Individuals show prolongation of the QT interval (QTc $\geq$ 460 ms) and/or documented *torsade de pointes*, ventricular fibrillation, cardiac arrest or aborted sudden death. Informed consent was obtained in accordance with local institutional review board guidelines. Phenotypic data were interpreted without knowledge of genotype. Sequence changes altering coding regions or predicted to affect splicing that were not detected in at least 400 control chromosomes were defined as mutations. No changes except known polymorphisms were detected in any of the genes in the control population. This does not exclude the possibility that some mutations are rare variants not associated with disease.

Example 2Mutational Analyses

To determine the spectrum of LQTS mutations, we used SSCP (Single Strand Conformation Polymorphism) and DNA sequence analyses to screen 262 unrelated individuals with LQTS. Seventeen primer pairs were used to screen *KVLQT1* (Splawski et al., 1998), twenty-one primer pairs were used for *HERG* (Splawski et al., 1998) and three primer pairs were used for *KCNE1* (Splawski et al., 1997a) and *KCNE2* (Abbott et al., 1999). Thirty-three primer pairs (Wang Q. et al., 1996b) were used in SSCP analysis to screen all *SCN5A* exons in 50 individuals with suspected abnormalities in  $I_{Na}$ . Exons 23-28, in which mutations were previously identified, were screened in all 262 individuals.

Gender, age, QTc and presence of symptoms are summarized in Table 1. The average age at ascertainment was 29 with a corrected QT interval of 492 ms. Seventy-five percent had a history of symptoms and females predominated with an ~ 2:1 ratio. Although the numbers were small, corrected QT intervals for individuals harboring *KCNE1* and *KCNE2* mutations were shorter at 457 ms.

Table 1

Age, QTc, Gender and Presence of Symptoms

Genotype	Age*, y (mean±SD)	Gender (F/M)	QTc, ms (mean±SD)	Symptoms†
<i>KVLQT1</i>	32 ± 19	32/23	493 ± 45	78%
<i>HERG</i>	31 ± 19	51/29	498 ± 48	71%
<i>SCN5A</i>	32 ± 24	8/6	511 ± 42	55%
<i>KCNE1</i>	43 ± 16	3/2	457 ± 25	40%
<i>KCNE2</i>	54 ± 20	3/0	457 ± 05	67%
unknown	25 ± 16	56/29	484 ± 46	81%
all	29 ± 19	173/89	492 ± 47	75%

\* - age at ascertainment

† - symptoms include syncope, cardiac arrest or sudden death

The SSCP analyses revealed many mutations. *KVLQT1* mutations associated with LQTS were identified in 52 individuals (Figure 1 and Table 2). Twenty of the mutations were novel. *HERG* mutations were identified in 68 LQTS individuals (Figure 2 and Table 3). Fifty-two of these mutations were novel. *SCN5A* mutations were identified in eight cases (Figure 3 and Table 4). Five of the mutations were novel. Three novel *KCNE1* mutations were identified (Figure 4 and Table 5) and three mutations were identified in *KCNE2* (Figure 5 and Table 6) (Abbott et al., 1999). None of the *KVLQT1*, *HERG*, *SCN5A*, *KCNE1* and *KCNE2* mutations was observed in 400 control chromosomes.

Table 2

Summary of All *KVLQT1* Mutations\*

Nucleotide Change†	Coding Effect	Position	Exon	Number of families‡	Study
del211-219	del71-73	N-terminus	1	1	Ackerman et al., 1999a
A332G †	Y111C	N-terminus	1	1	This

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Nucleotide Change <sup>†</sup>	Coding Effect	Position	Exon	Number of families <sup>‡</sup>	Study
del451-452	A150fs/132	S2	2	1 JLN	Chen Q. et al., 1999
T470G	F157C	S2	1	1	Larsen et al., 1999a
G477+1A	M159sp	S2	2	1 JLN, 1 UK	This; Donger et al., 1997
G477+5A	M159sp	S2	1	1	Ackerman et al., 1999b
G478A †	E160K	S2	3	1	This
del500-502	F167W/del G168	S2	3	1	Wang Q. et al., 1996a
G502A	G168R	S2	3	7	This; Splawski et al., 1998; Donger et al., 1997
C520T	R174C	S2/S3	3	1	Donger et al., 1997
G521A †	R174H	S2/S3	3	1	This
G532A	A178T	S2/S3	3	1	Tanaka et al., 1997
G532C	A178P	S2/S3	3	1	Wang Q. et al., 1996a
G535A †	G179S	S2/S3	3	1	This
A551C	Y184S	S2/S3	3	2	This; Jongbloed et al., 1999
G565A	G189R	S2/S3	3	3	Wang Q. et al., 1996a; Jongbloed et al., 1999
insG567- 568	G189fs/94	S2/S3	3	1 (RW + JLN)	Splawski et al., 1997b
G569A	R190Q	S2/S3	3	2	Splawski et al., 1998; Donger et al., 1997
del572-576	L191fs/90	S2/S3	3	1 JLN, 1 RW 2 (JLN + RW)	Tyson et al., 1997; Ackerman et al., 1999b

25	Nucleotide Change <sup>†</sup>	Coding Effect	Position	Exon	Number of families <sup>†</sup>	Study
5	G580C †	A194P	S2/S3	3	1	This
	C674T	S225L	S4	4	2	This; Priori et al., 1999
	G724A	D242N	S4/S5	5	1	Itoh et al., 1998b
	C727T †	R243C	S4/S5	5	2	This
	G728A	R243H	S4/S5	5	1 JLN	Saارين et al., 1998
10	T742C †	W248R	S4/S5	5	1	This
	T749A	L250H	S4/S5	5	1	Itoh et al., 1998a
	G760A	V254M	S4/S5	5	4	This; Wang Q. et al., 1996a; Donger et al., 1997
	G781A	E261K	S4/S5	6	1	Donger et al., 1997
	T797C †	L266P	S5	6	1	This
15	G805A	G269S	S5	6	1	Ackerman et al., 1999b
	G806A	G269D	S5	6	3	This; Donger et al., 1997
	C817T	L273F	S5	6	2	This; Wang Q. et al., 1996a
	A842G	Y281C	S5	6	1	Priori et al., 1999
	G898A	A300T	S5/Pore	6	1	Priori et al., 1998
20	G914C	W305S	Pore	6	1 JLN	Chouabe et al., 1997
	G916A	G306R	Pore	6	1	Wang Q. et al, 1996a
	del921-(921+2)	V307sp	Pore	6	1	Li et al., 1998
	G921+1T †	V307sp	Pore	6	1	This
	A922-2C †	V307sp	Pore	7	1	This
	G922-1C	V307sp	Pore	7	1	Murray et al., 1999
	C926G	T309R	Pore	7	1	Donger et al., 1997

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Nucleotide Change <sup>†</sup>	Coding Effect	Position	Exon	Number of families <sup>‡</sup>	Study
G928A †	V310I	Pore	7	1	This
C932T	T311I	Pore	7	1	Saarinen et al., 1998
C935T	T312I	Pore	7	2	This; Wang Q. et al., 1996a
C939G	I313M	Pore	7	1	Tanaka et al., 1997
G940A	G314S	Pore	7	7	Splawski et al., 1998; Russell et al., 1996; Donger et al., 1997; Jongbloed et al., 1999; Itoh et al., 1998b
A944C	Y315S	Pore	7	3	Donger et al., 1997; Jongbloed et al., 1999
A944G	Y315C	Pore	7	2	Priori et al., 1999; Splawski et al., 1998
G949A	D317N	Pore	7	2	Wollnik et al., 1997; Saarinen et al., 1998
G954C	K318N	Pore	7	1	Splawski et al., 1998
C958G	P320A	Pore	7	1	Donger et al., 1997
G973A	G325R	S6	7	4	This; Donger et al., 1997; Tanaka et al., 1997
del1017- 1019	delF340	S6	7	2	This; Ackerman et al., 1998
C1022A	A341E	S6	7	5	This; Wang Q. et al., 1996a; Berthet et al., 1999

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	Nucleotide Change <sup>†</sup>	Coding Effect	Position	Exon	Number of families <sup>‡</sup>	Study
	C1022T	A341V	S6	7	7	This; Wang Q. et al., 1996a; Russell et al., 1996; Donger et al., 1997; Li et al., 1998
	C1024T	L342F	S6	7	1	Donger et al., 1997
	C1031T	A344V	S6	7	1	Donger et al., 1997
	G1032A	A344sp	S6	7	9	This; Kanters, 1998; Li et al., 1998; Ackerman et al., 1999b; Murray et al., 1999
5	G1032C	A344sp	S6	7	1	Murray et al., 1999
	G1033C	G345R	S6	8	1	van den Berg et al., 1997
	G1034A	G345E	S6	8	1	Wang Q. et al., 1996a
	C1046G †	S349W	S6	8	1	This
	T1058C	L353P	S6	8	1	Splawski et al., 1998
10	C1066T †	Q356X	C-terminus	8	1	This
	C1096T	R366W	C-terminus	8	1	Splawski et al., 1998
	G1097A †	R366Q	C-terminus	8	1	This
	G1097C	R366P	C-terminus	8	1	Tanaka et al., 1997
	G1111A	A371T	C-terminus	8	1	Donger et al., 1997
15	T1117C	S373P	C-terminus	8	1	Jongbloed et al., 1999
	C1172T †	T391I	C-terminus	9	1	This
	T1174C	W392R	C-terminus	9	1	Jongbloed et al., 1999
	C1343G †	P448R	C-terminus	10	2	This
	C1522T	R518X	C-terminus	12	1 JLN, 3 RW	This; Larsen et al., 1999

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Nucleotide Change <sup>†</sup>	Coding Effect	Position	Exon	Number of families <sup>‡</sup>	Study
G1573A	A525T	C-terminus	12	1	Larsen et al., 1999b
C1588T †	Q530X	C-terminus	12	1 JLN, 1 RW	This
C1615T	R539W	C-terminus	13	1	Chouabe et al., 1997
del6/ins7	E543fs/107	C-terminus	13	1 JLN	Neyroud et al., 1997
C1663T	R555C	C-terminus	13	3	Donger et al., 1997
C1697T †	S566F	C-terminus	14	3	This
C1747T †	R583C	C-terminus	15	1	This
C1760T	T587M	C-terminus	15	1 JLN, 1 RW	Donger et al., 1997; Itoh et al., 1998b
G1772A	R591H	C-terminus	15	1	Donger et al., 1997
G1781A †	R594Q	C-terminus	15	3	This
del1892-1911	P630fs/13	C-terminus	16	1 JLN	Donger et al., 1997
insC1893-1894	P631fs/19	C-terminus	16	1	Donger et al., 1997

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\* - ins denotes insertion; del denotes deletion; sp denotes the last unaffected amino acid before the predicted splice mutation; fs denotes the last amino acid unaffected by a frameshift, following fs is the number of amino acids before termination; X denotes a stop codon occurred.

† - denotes novel mutation

‡ - Number of Romano-Ward families unless otherwise indicated (UK - unknown)

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Table 3

Summary of All *HERG* Mutations\*

	Nucleotide Change	Coding Effect	Position	Exon	Number of RW Families	Study
5	C87A †	F29L	N-terminus	2	1	This
	A98C †	N33T	N-terminus	2	2	This
	C132A †	C44X	N-terminus	2	1	This
	G140T †	G47V	N-terminus	2	1	This
	G157C †	G53R	N-terminus	2	1	This
10	G167A †	R56Q	N-terminus	2	1	This
	T196G †	C66G	N-terminus	2	1	This
	A209G †	H70R	N-terminus	2	2	This
	C215A †	P72Q	N-terminus	2	2	This
	del221-251 †	R73fs/31	N-terminus	2	1	This
15	G232C †	A78P	N-terminus	2	1	This
	dupl234-250†	A83fs/37	N-terminus	2	1	This
	C241T †	Q81X	N-terminus	2	1	This
	T257G †	L86R	N-terminus	2	1	This
	insC422-423†	P141fs/2	N-terminus	3	1	This
20	insC453-454†	P151fs/179	N-terminus	3	1	This
	dupl558-600	L200fs/144	N-terminus	4	1	Hoorntje et al., 1999
	insC724-725†	P241fs/89	N-terminus	4	1	This
	del885 †	V295fs/63	N-terminus	4	1	This
	C934T †	R312C	N-terminus	5	1	This
25	C1039T †	P347S	N-terminus	5	1	This
	G1128A †	Q376sp	N-terminus	5	1	This

	Nucleotide Change	Coding Effect	Position	Exon	Number of RW Families	Study
5	A1129-2G †	Q376sp	N-terminus	6	1	This
	del1261	Y420fs/12	S1	6	1	Curran et al., 1995
	C1283A	S428X	S1/S2	6	1	Priori et al., 1999
	C1307T	T436M	S1/S2	6	1	Priori et al., 1999
	A1408G	N470D	S2	6	1	Curran et al., 1995
	C1421T	T474I	S2/S3	6	1	Tanaka et al., 1997
	C1479G	Y493X	S2/S3	6	1	Itoh et al., 1998a
10	del1498-1524	del500-508	S3	6	1	Curran et al., 1995
	G1592A †	R531Q	S4	7	1	This
	C1600T	R534C	S4	7	1	Itoh et al., 1998a
	T1655C †	L552S	S5	7	1	This
	delT1671	T556fs/7	S5	7	1	Schulze-Bahr et al., 1995
	G1672C	A558P	S5	7	1	Jongbloed et al., 1999
	G1681A	A561T	S5	7	4	This; Dausse et al., 1996
15	C1682T	A561V	S5	7	4	This; Curran et al., 1995; Priori et al., 1999
	G1714C	G572R	S5/Pore	7	1	Larsen et al., 1999a
	G1714T	G572C	S5/Pore	7	1	Splawski et al., 1998
	C1744T	R582C	S5/Pore	7	1	Jongbloed et al., 1999
	G1750A †	G584S	S5/Pore	7	1	This
	G1755T †	W585C	S5/Pore	7	1	This
	A1762G	N588D	S5/Pore	7	1	Splawski et al., 1998
20	T1778C †	I593T	S5/Pore	7	1	This
	T1778G	I593R	S5/Pore	7	1	Benson et al., 1996
	G1801A	G601S	S5/Pore	7	1	Akimoto et al., 1998

	<b>Nucleotide Change</b>	<b>Coding Effect</b>	<b>Position</b>	<b>Exon</b>	<b>Number of RW Families</b>	<b>Study</b>
5	G1810A	G604S	S5/Pore	7	2	This; Jongbloed et al., 1999
	G1825A †	D609N	S5/Pore	7	1	This
	T1831C	Y611H	S5/Pore	7	1	Tanaka et al., 1997
	T1833 (A or G)	Y611X	S5/Pore	7	1	Schulze-Bahr et al., 1995
	G1834T	V612L	Pore	7	1	Satler et al., 1998
10	C1838T	T613M	Pore	7	4	This; Jongbloed et al., 1999
	C1841T	A614V	Pore	7	6	Priori et al., 1999; Splawski et al., 1998; Tanaka et al., 1997; Satler et al., 1998
	C1843G †	L615V	Pore	7	1	This
	G1876A †	G626S	Pore	7	1	This
	C1881G †	F627L	Pore	7	1	This
15	G1882A	G628S	Pore	7	2	This; Curran et al., 1995
	A1885G	N629D	Pore	7	1	Satler et al., 1998
	A1886G	N629S	Pore	7	1	Satler et al., 1998
	C1887A	N629K	Pore	7	1	Yoshida et al., 1999
	G1888C	V630L	Pore	7	1	Tanaka et al., 1997
20	T1889C	V630A	Pore	7	1	Splawski et al., 1998
	C1894T †	P632S	Pore	7	1	This
	A1898G	N633S	Pore	7	1	Satler et al., 1998
	A1912G †	K638E	S6	7	1	This
	del1913-1915†	delK638	S6	7	1	This

	<b>Nucleotide Change</b>	<b>Coding Effect</b>	<b>Position</b>	<b>Exon</b>	<b>Number of RW Families</b>	<b>Study</b>
5	C1920A	F640L	S6	7	1	Jongbloed et al., 1999
	A1933T †	M645L	S6	7	1	This
	del1951-1952	L650fs/2	S6	8	1	Itoh et al., 1998a
	G2044T †	E682X	S6/cNBD	8	1	This
	C2173T	Q725X	S6/cNBD	9	1	Itoh et al., 1998a
10	insT2218- 2219 †	H739fs/63	S6/cNBD	9	1	This
	C2254T †	R752W	S6/cNBD	9	1	This
	dupl2356-2386	V796fs/22	cNBD	9	1	Itoh et al., 1998a
	del2395 †	I798fs/10	cNBD	9	1	This
	G2398+1C	L799sp	cNBD	9	2	This; Curran et al., 1995
15	T2414C †	F805S	cNBD	10	1	This
	T2414G †	F805C	cNBD	10	1	This
	C2453T	S818L	cNBD	10	1	Berthet et al., 1999
	G2464A	V822M	cNBD	10	2	Berthet et al., 1999; Satler et al., 1996
	C2467T †	R823W	cNBD	10	2	This
20	A2582T †	N861I	C-terminus	10	1	This
	G2592+1A	D864sp	C-terminus	10	2	This; Berthet et al., 1999
	del2660 †	K886fs/85	C-terminus	11	1	This
	C2750T †	P917L	C-terminus	12	1	This
	del2762 †	R920fs/51	C-terminus	12	1	This
25	C2764T †	R922W	C-terminus	12	1	This
	insG2775- 2776 †	G925fs/13	C-terminus	12	1	This
	del2906 †	P968fs/4	C-terminus	12	1	This

Nucleotide Change	Coding Effect	Position	Exon	Number of RW Families	Study
del2959-2960†	P986fs/130	C-terminus	12	1	This
C3040T †	R1014X	C-terminus	13	2	This
del3094 †	G1031fs/24	C-terminus	13	1	This
insG3107-3108	G1036fs/82	C-terminus	13	1	Berthet et al., 1999
insC3303-3304 †	P1101fs	C-terminus	14	1	This

\* - all characters same as in Table 2

Table 4

Summary of All SCN5A Mutations

Nucleotide Change	Coding Effect	Position	Exon	Number of RW Families	Study
G3340A †	D1114N	DII/DIII	18	1	This
C3911T	T1304M	DIII/S4	22	1	Wattanasirichaigoon et al., 1999
A3974G	N1325S	DIII/S4/S5	23	1	Wang et al., 1995b
C4501G †	L1501V	DIII/DIV	26	1	This
del4511-4519	del1505 - 1507	DIII/DIV	26	4	Wang et al., 1995a; Wang et al., 1995b
del4850-4852 †	delF1617	DIV/S3/S4	28	1	This
G4868A	R1623Q	DIV/S4	28	2	This; Makita et al., 1998
G4868T †	R1623L	DIV/S4	28	1	This

Nucleotide Change	Coding Effect	Position	Exon	Number of RW Families	Study
G4931A	R1644H	DIV/S4	28	2	This; Wang et al., 1995b
C4934T	T1645M	DIV/S4	28	1	Wattanasirichaigoon et al., 1999
G5350A †	E1784K	C-terminus	28	2	This; Wei et al., 1999
G5360A †	S1787N	C-terminus	28	1	This
A5369G	D1790G	C-terminus	28	1	An et al., 1998
insTGA 5385-5386	insD1795 -1796	C-terminus	28	1	Bezzina et al., 1999

\* - all characters same as in Table 2. Fifty individuals with suspected abnormalities in  $I_{Na}$  were screened for all *SCN5A* exons. All individuals were screened for exons 23-28.

Table 5

Summary of All *KCNE1* Mutations\*

Nucleotide Change	Coding Effect	Position	Exon	Number of Families	Study
C20T	T7I	N-terminus	3	1 JLN	Schulze-Bahr et al., 1997
G95A †	R32H	N-terminus	3	1	This
G139T	V47F	S1	3	1 JLN	Bianchi et al., 1999
TG151-152AT	L51H	S1	3	1 JLN	Bianchi et al., 1999
A172C/TG 176-177CT	TL58-59PP	S1	3	1 JLN	Tyson et al., 1997
C221T	S74L	C-terminus	3	1	Splawski et al., 1997a

Nucleotide Change	Coding Effect	Position	Exon	Number of Families	Study
G226A	D76N	C-terminus	3	1 JLN, 1 RW, 1 (JLN + RW)	Splawski et al., 1997a; Tyson et al., 1997; Duggal et al., 1998
T259C	W87R	C-terminus	3	1	Bianchi et al., 1999
C292T †	R98W	C-terminus	3	1	This
C379A †	P127T	C-terminus	3	1	This

\* - all characters same as in Table 2

Table 6

Summary of All *KCNE2* Mutations

Nucleotide Change	Coding Effect	Position	Exon	Number of Families	Study
C25G	Q9E	N-terminus	1	1	Abbott et al., 1999
T161T	M54T	S1	1	1	Abbott et al., 1999
T170C	I57T	S1	1	1	Abbott et al., 1999

Table 7

Mutations by Type

Type	<i>KVLQT1</i>	<i>HERG</i>	<i>SCN5A</i>	<i>KCNE1</i>	<i>KCNE2</i>	Total
Missense	59	52	9	5	3	128
Nonsense	6	5	0	0	0	11
AA deletion*	2	2	5	0	0	9
Frameshift	1	16	0	0	0	17
Splice	7	5	0	0	0	12
Total	75	80	14	5	3	177

\* - AA denotes amino acid

Table 8

Mutations by Position

Gene Protein Position	<i>KVLQT1</i> KVLQT1	<i>HERG</i> HERG	<i>SCN5A</i> SCN5A	<i>KCNE1</i> minK	<i>KCNE2</i> MiRP1	Total
Extracellular	0	7	1	1	1	10
Transmembrane	33	13	5	0	2	53
Pore	9	12	0	N/A	N/A	21
Intracellular	33	48	8	4	0	93
Total	75	80	14	5	3	177

While the invention has been disclosed in this patent application by reference to the details of preferred embodiments of the invention, it is to be understood that the disclosure is intended in an illustrative rather than in a limiting sense, as it is contemplated that modifications will readily occur to those skilled in the art, within the spirit of the invention and the scope of the appended claims.



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